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14. ABSTRACT
Steroid Receptor Coactivator-3 (SRC-3) is a transcriptional coactivator that promotes proliferation of prostate cancer (CaP) cells. Although SRC-3 is highly expressed in advanced CaP, its role in PTEN deficiency-promoted castration resistant CaP (CRPC) is unknown. We found elevated SRC-3 in human CRPC and in PTEN-negative human CaP. Patients with high SRC-3 and no PTEN exhibit decreased recurrence-free survival. We generated Pten3CKO mice in which floxed Pten and SRC-3 genes are specifically and concomitantly deleted in prostate epithelial cells (PECs). We compared tumor mass, histology and biomarkers in these Pten3CKO mice versus PtenCKO control mice in which only floxed Pten alleles are deleted in PECs. Deletion of SRC-3 impaired cellular proliferation, causing reduced tumor size. Interestingly, castration of PtenCKO control mice significantly increased tumor aggressiveness over time versus their non-castrated counterparts, as indicated by increased proliferation, de-differentiation and reactive stroma. Remarkably, SRC-3 ablation in Pten3CKO mice reversed all the changes comprising this aggressive phenotype. Versus controls, Pten3CKO tumors in castrated mice had decreased phospho-Akt, S6 kinase and phosphorylated S6, which are mediators of cell growth and proliferation. In addition, these tumors seem more differentiated as evidenced by higher levels of Fkbp5, an AR-responsive gene that inhibits Akt signaling. These tumors also had lower levels of some androgen-repressed genes like cyclin E2 and MMP10. These results reveal SRC-3 promotes CRPC by inducing proliferation, de-differentiation, and stromal reactivity possibly through the Akt/S6K pathway. These features make SRC-3 a putative target for abrogating CRPC progression.

15. SUBJECT TERMS- Prostate cancer, Castration-resistant prostate cancer, steroid-receptor coactivator-3

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INTRODUCTION:

Prostate cancer affects one in six American men and is the second leading cause of cancer death in this population (ACS, 2010). Advanced disease, in which cancer invades adjacent structures and gains metastatic capacity, is the primary cause of prostate cancer-related mortality. Steroid receptor coactivator 3 (SRC-3) is a nuclear receptor coactivator that is important for growth of endocrine tissues (1). SRC-3 enhances proliferation of prostate cancer cell lines in a cell-autonomous manner and its expression is highly correlated with aggressiveness of human prostate cancer tumor samples (2,3). In addition, prostate cancer cell line studies have revealed that SRC-3 serves as a coactivator for AP1 transcription factor, promoting cell proliferation through the IGF/Akt pathway (4). Here we use animal models to study the function of SRC-3 in prostate cancer and ascertain the role of SRC-3 in a cell-type specific manner by employing the lox-Cre knockout system. In the first part of my research funding period, I aimed to determine whether SRC-3 promotes prostate tumor progression in cancer derived from luminal epithelial cells (LECs) by simultaneously deleting the Pten and SRC-3 genes in the LECs of the mouse prostate. I also performed castration experiments on these mice to assess the role of SRC-3 in the development of castration-resistant prostate cancer. During the second part of my research funding period, I have completed the research, prepared a manuscript, and submitted the manuscript to *Cancer Research*. I also wrote a review on the potential of targeting SRC-3 as cancer therapeutic drug. The review is published in the journal *Expert Opinions on Therapeutic Target*. In the second part, I aimed to ascertain whether SRC-3 promotes prostate cancer progression in tumors arising from basal cell progenitors by simultaneously deleting SRC-3 and Pten genes in the basal cells of mouse prostate using a similar strategy.

BODY:

1. **Research Training:**

During the last year, I advanced my research training in several respects. First, I wrote a review article on the general role of SRC-3 in cancer and specific recent efforts at developing small molecule SRC-3 inhibitors. The review is now published in the journal Expert Opinion on Therapeutic Targets. In addition, I wrote a manuscript on the primary study demonstrating a role for SRC-3 in the progression of castration-resistant prostate cancer. This manuscript was provisionally accepted to Cancer Research after the first round of review. I am now making cosmetic changes to the article requested by the editors prior to its inclusion in the journal. The review article, as well as the paper figures are attached in the appendix.

2. **Research Project:**

Specific Aim 2: Ascertain whether SRC-3 promotes prostate cancer progression in tumors arising from basal cell progenitors by simultaneously deleting floxed SRC-3 and PTEN genes in BCs via p63CreERT2

Task 4: Generate experimental ($pten^{f/f}$ /$SRC\text{-}3^{f/f}$ /p63CreERT2) and control mice ($pten^{f/f}$/ p63CreERT2) mice

We backcrossed the $Pten^{f/f}$, $SRC\text{-}3^{f/f}$ and p63CreERT2 mice to FVB background for six generations before we generated the experimental and control mice. I generated the experimental and control mice at the beginning of second year. The mice were injected with 100mg/kg tamoxifen for 7 consecutive days at age of six weeks to induce deletion of the genes. These mice were sacrificed at 18 weeks and the prostate samples were collected for histology assessment.

Task 5: Assess tumor initiation, progression, and metastasis

I collected the tumor samples and found that tumorigenesis was minimal in these animals. At this point, I suspended the study on the basis that this genetic background is not amenable for the development of pten-mediated prostate cancer. Future studies in the Xu lab will employ other mouse strains to evaluate this tumor type.

KEY RESEARCH ACCOMPLISHMENTS

- We found that deletion of SRC-3 significantly reduces tumor size and cellular proliferation in castration-resistant prostate cancer and deletion of SRC-3 reverses castration-induced changes in tumor cell type and stromal reactivity. A manuscript based on this research study was prepared and submitted to Cancer Research journal. The manuscript was provisionally accepted

REPORTABLE OUTCOMES:

Publications

Tien J.C., Liu Z., Liao L., Wang F., Xu Y., Wu Y., Zhou N., Ittmann. M., Xu J (2013). "SRC-3 Is Required for the Development of Castration-resistant Prostate Cancer." Provisionally accepted Cancer Research

Tien, J. C. and J. Xu (2012). "Steroid receptor coactivator-3 as a potential molecular target for cancer therapy." Expert Opin Ther Targets **16**(11): 1085-1096.

Walsh, C. A., L. Qin, **J. C. Tien**, L. S. Young and J. Xu (2012). "The function of steroid receptor coactivator-1 in normal tissues and cancer." Int J Biol Sci **8**(4): 470-485.

CONCLUSION:

Here, I simultaneously deleted Pten and SRC-3 using the ARR2PBi-Cre, which is expressed principally in prostatic luminal epithelial cells. From the results obtained, I conclude that inhibition of SRC-3 in the setting of Pten deletion yields smaller prostate tumor size with a more basal-like cell phenotype. This indicates SRC-3 plays an oncogenic role in tumors derived from luminal cells and that without SRC-3, mitotic ability of LEC-like tumor cells is impaired. During the course of studying the function of SRC-3 in castration-resistant prostate cancer, we found that although androgen deprivation shrunk the size of the tumor, the reduced level of testosterone accelerates prostate tumorigenesis, making castrated Pten-null tumors more aggressive and less differentiated with increased stromal reactivity. Amazingly, the androgen-deprived tumor phenotype is dependent on SRC-3, as SRC-3 deletion results in almost complete reversal of all castration-induced changes. SRC-3 deletion also yields decrease in S6 kinase, a mediator of cellular translational output. Therefore, SRC-3 may mediate translational pathway that contributes to castration-resistant prostate cancer. Critically, we identified relationships between SRC-3 overexpression and androgen independence, as well as upregulation of the aforementioned signaling pathway in human prostate cancer samples. Unfortunately, attempts to extend our knowledge of SRC-3 function to its impact on basal cell-derived cancer were hampered by the inability of our mouse model to develop adequate tumors. Nonetheless, our work to date has defined SRC-3 as a critical mediator of advanced and castration-resistant prostate cancer. Design of SRC-3 inhibitor may be a novel therapeutic strategy for treating both tumor types.

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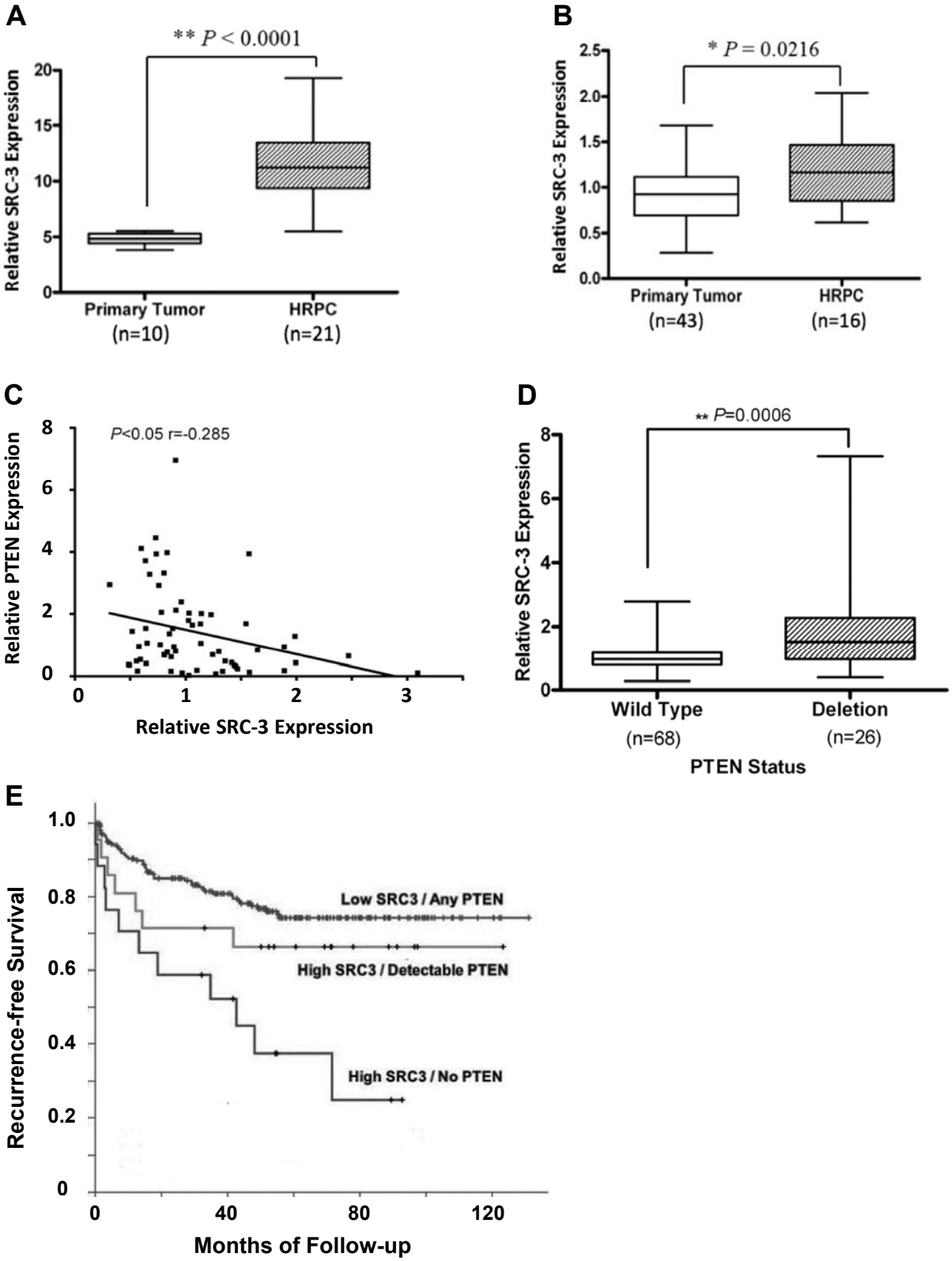

A
** P < 0.0001
Relative SRC-3 Expression
20
15
10
5
0
Primary Tumor
(n=10)
HRPC
(n=21)
B
* P = 0.0216
Relative SRC-3 Expression
2.5
2.0
1.5
1.0
0.5
0.0
Primary Tumor
(n=43)
HRPC
(n=16)
C
P<0.05 r=-0.285
Relative PTEN Expression
8
6
4
2
0
0
1
2
3
Relative SRC-3 Expression
D
** P=0.0006
Relative SRC-3 Expression
8
6
4
2
0
Wild Type
(n=68)
Deletion
(n=26)
PTEN Status
E
Recurrence-free Survival
1.0
0.8
0.6
0.4
0.2
0
Low SRC3 / Any PTEN
High SRC3 / Detectable PTEN
High SRC3 / No PTEN
0
40
80
120
Months of Follow-up

Fig. 1

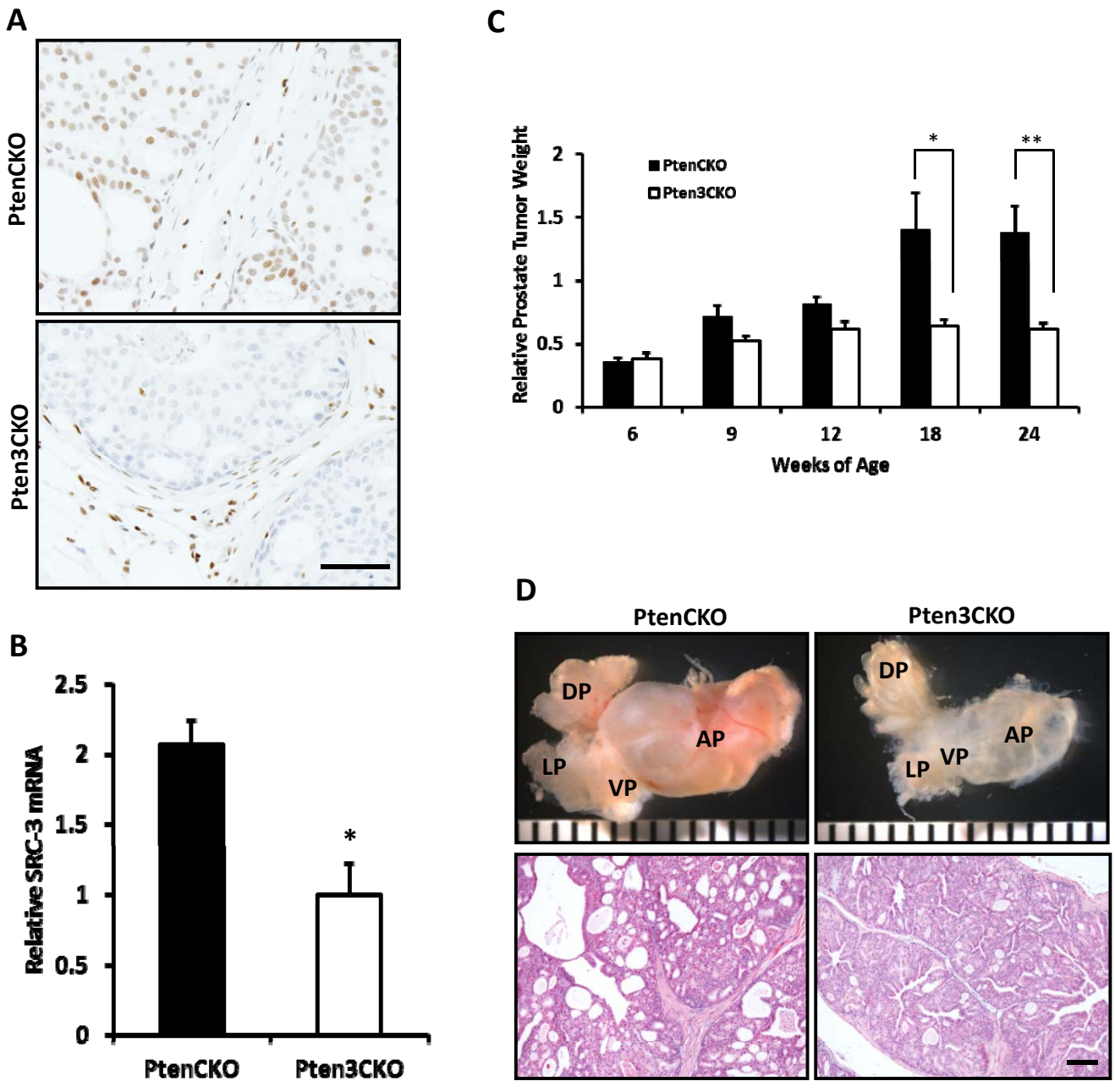

A
PtenCKO
Pten3CKO
B
Relative SRC-3 mRNA
2.5
2
1.5
1
0.5
0
*
PtenCKO
Pten3CKO
C
Relative Prostate Tumor Weight
2
1.5
1
0.5
0
PtenCKO
Pten3CKO
*
**
6
9
12
18
24
Weeks of Age
D
PtenCKO
Pten3CKO
DP
AP
LP
VP
DP
AP
LP
VP

Fig. 2

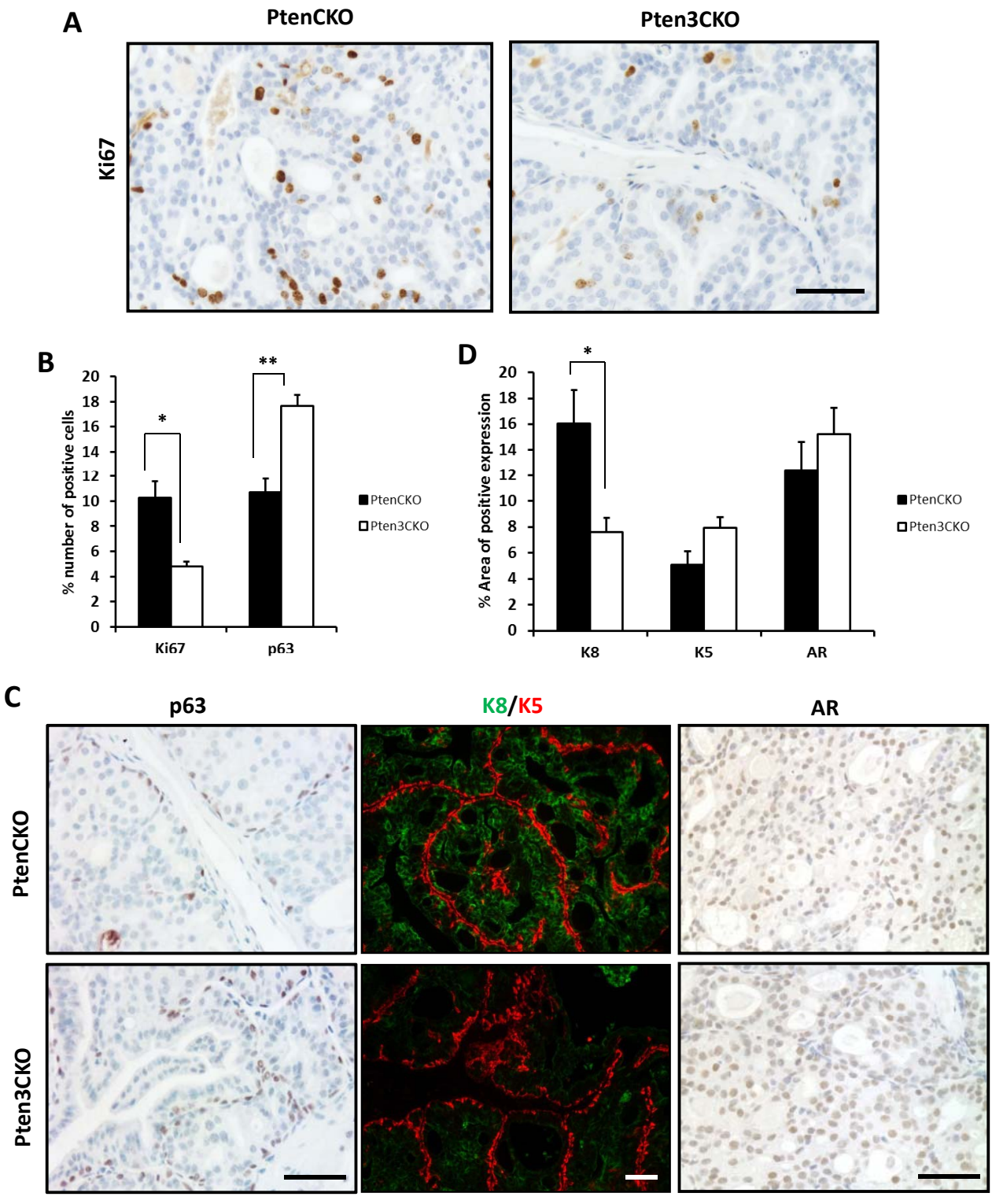

A
PtenCKO
Pten3CKO
Ki67
B
% number of positive cells
*
**
PtenCKO
Pten3CKO
Ki67
p63
D
% Area of positive expression
*
PtenCKO
Pten3CKO
K8
K5
AR
C
p63
K8/K5
AR
PtenCKO
Pten3CKO

Fig. 3

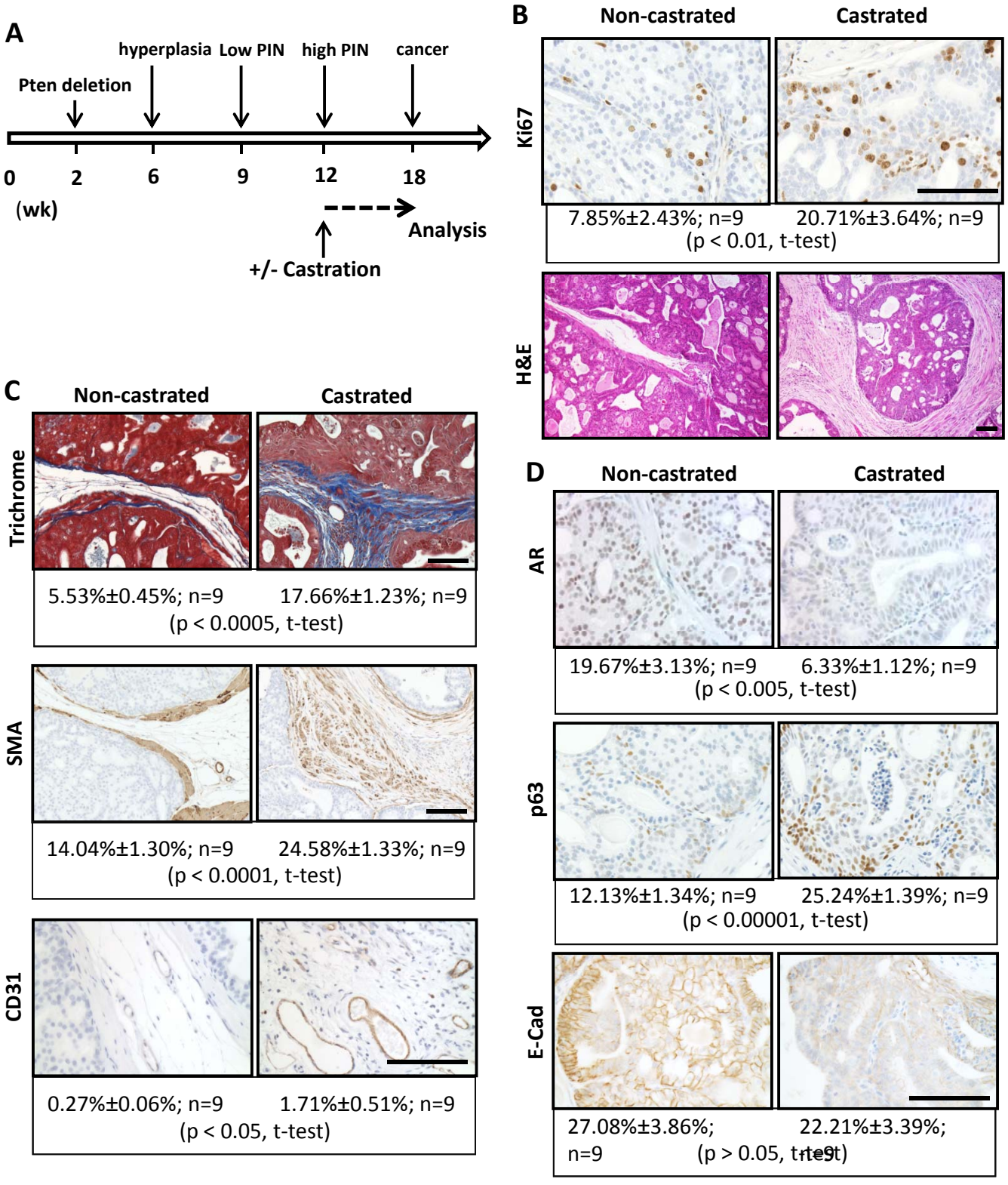
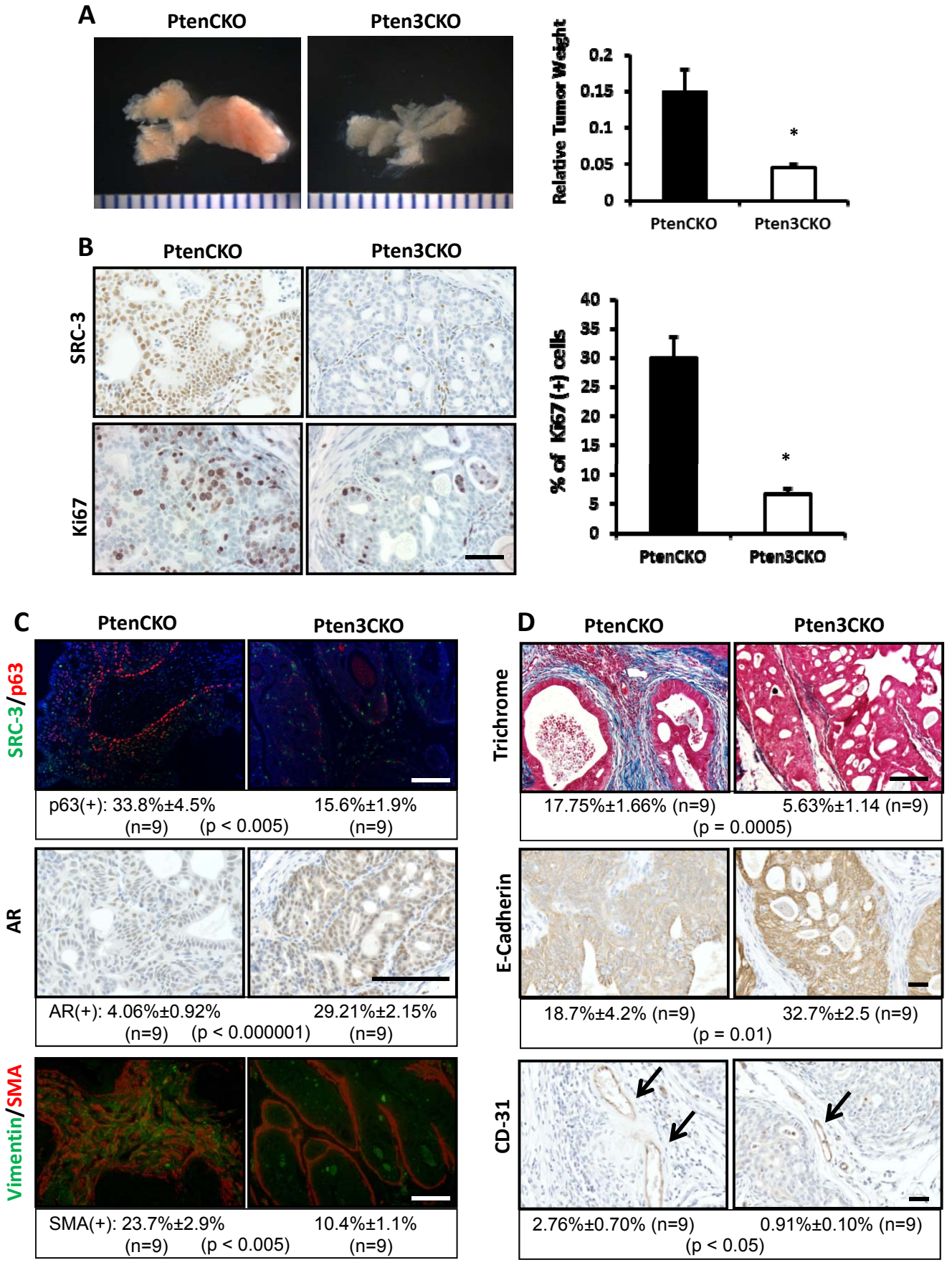

A
hyperplasia
Low PIN
high PIN
cancer
Pten deletion
0
2
6
9
12
18
(wk)
Analysis
+/- Castration
B
Non-castrated
Castrated
Ki67
7.85%±2.43%; n=9
20.71%±3.64%; n=9
(p < 0.01, t-test)
H&E
C
Non-castrated
Castrated
Trichrome
5.53%±0.45%; n=9
17.66%±1.23%; n=9
(p < 0.0005, t-test)
SMA
14.04%±1.30%; n=9
24.58%±1.33%; n=9
(p < 0.0001, t-test)
CD31
0.27%±0.06%; n=9
1.71%±0.51%; n=9
(p < 0.05, t-test)
D
Non-castrated
Castrated
AR
19.67%±3.13%; n=9
6.33%±1.12%; n=9
(p < 0.005, t-test)
p63
12.13%±1.34%; n=9
25.24%±1.39%; n=9
(p < 0.00001, t-test)
E-Cad
27.08%±3.86%;
n=9
22.21%±3.39%;
(p > 0.05, t-test)

Fig. 4


A
PtenCKO
Pten3CKO
Relative Tumor Weight
0.2
0.15
0.1
0.05
0
*
PtenCKO
Pten3CKO
B
PtenCKO
Pten3CKO
SRC-3
Ki67
% of Ki67 (+) cells
40
35
30
25
20
15
10
5
0
*
PtenCKO
Pten3CKO
C
PtenCKO
Pten3CKO
SRC-3/p63
p63(+): 33.8%±4.5% (n=9)
(p < 0.005)
15.6%±1.9% (n=9)
AR
AR(+): 4.06%±0.92% (n=9)
(p < 0.000001)
29.21%±2.15% (n=9)
Vimentin/SMA
SMA(+): 23.7%±2.9% (n=9)
(p < 0.005)
10.4%±1.1% (n=9)
D
PtenCKO
Pten3CKO
Trichrome
17.75%±1.66% (n=9)
(p = 0.0005)
5.63%±1.14 (n=9)
E-Cadherin
18.7%±4.2% (n=9)
(p = 0.01)
32.7%±2.5 (n=9)
CD-31
2.76%±0.70% (n=9)
(p < 0.05)
0.91%±0.10% (n=9)

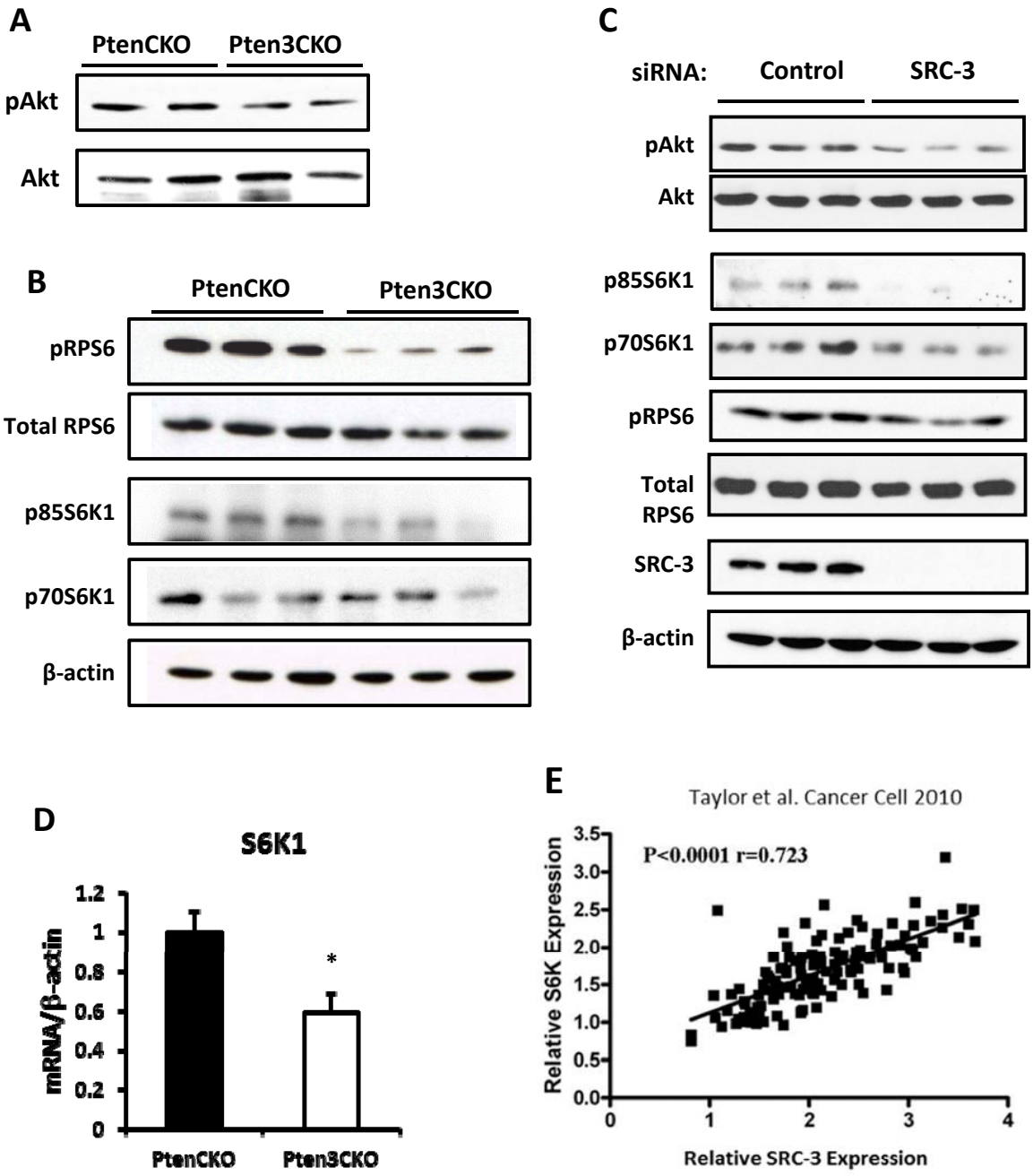

A
PtenCKO
Pten3CKO
pAkt
Akt
B
PtenCKO
Pten3CKO
pRPS6
Total RPS6
p85S6K1
p70S6K1
β-actin
C
siRNA:
Control
SRC-3
pAkt
Akt
p85S6K1
p70S6K1
pRPS6
Total RPS6
SRC-3
β-actin
D
S6K1
mRNA/β-actin
1.2
1
0.8
0.6
0.4
0.2
0
*
PtenCKO
Pten3CKO
E
Taylor et al. Cancer Cell 2010
P<0.0001 r=0.723
Relative S6K Expression
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
Relative SRC-3 Expression

Fig. 6

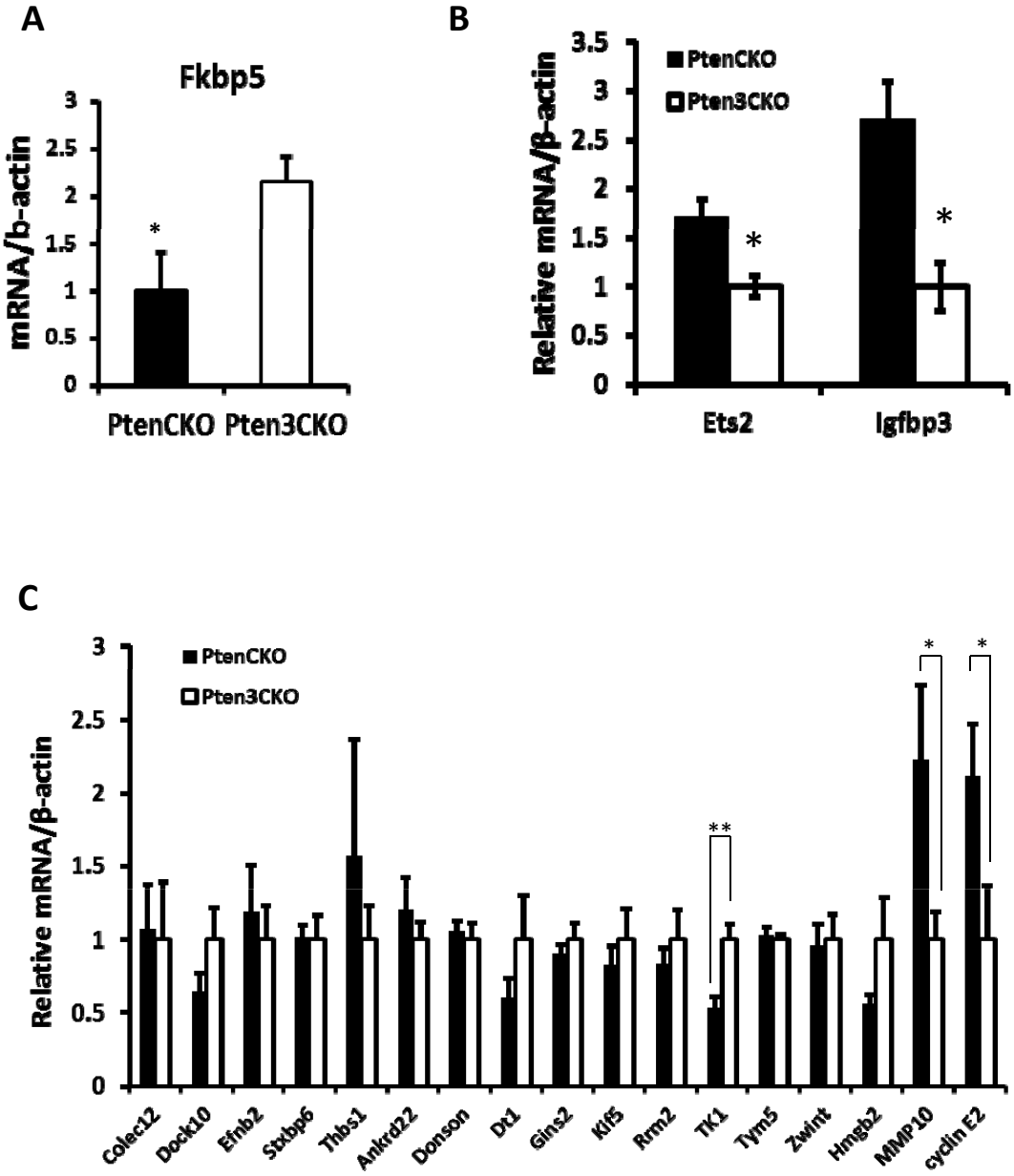

A
Fkbp5
mRNA/b-actin
3
2.5
2
1.5
1
0.5
0
*
PtenCKO
Pten3CKO
B
Relative mRNA/β-actin
3.5
3
2.5
2
1.5
1
0.5
0
PtenCKO
Pten3CKO
*
*
Ets2
Igfbp3
C
Relative mRNA/β-actin
3
2.5
2
1.5
1
0.5
0
PtenCKO
Pten3CKO
**
*
*
Colec12
Dock10
Efnb2
Stxbp6
Thbs1
Ankrd22
Donson
Dtl
Gins2
Klf5
Rrm2
TK1
Tym5
Zwint
Hmgb2
MMP10
cyclin E2

Fig. 7

FIGURE LEGENDS

Fig. 1. SRC-3 expression is elevated in human hormone refractory prostate cancer (HRPC) and negatively correlated with PTEN expression and recurrence-free survival. ***A*** and ***B.*** *SRC-3* expression in human HRPCs and primary prostate tumors. The mRNA expression data sets by Chandra et al. (Panel A) and by Tomlins et al. (Panel B) were downloaded from Oncomine. Data sets in each panel were from the same study source and were statistically analyzed by Mann-Whitney U test. ***C.*** Pearson's correlation analysis of the data set by Tomlins et al. (n=59) revealed a negative correlation between *SRC-3* and *PTEN* expression in human prostate tumors. ***D.*** The relative expression levels of *SRC-3* mRNA in human prostate tumors with *PTEN* wild type and *PTEN* deletion. The expression data sets were from Oncomine (Grasso et al. 2012). ***E.*** Patients with prostate tumors expressing high SRC-3 and no PTEN exhibit poor recurrence-free survival. Based on immunostaining scores, patients were divided into high SRC-3 with no PTEN (n=17), high SRC-3 with detectable PTEN (n=21) and low SRC-3 with any levels of PTEN (n=214), and data sets were analyzed using Kaplan-Meier Estimator. The p values are indicated in the text.

Fig. 2. Specific knockout of SRC-3 in PECs reduced Pten deletion-induced prostate tumor growth. ***A.*** Immunohistochemistry of SRC-3 (brown) on the prostate sections with PIN lesions prepared from 12-week-old PtenCKO and Pten3CKO mice. Note the negative SRC-3 immunostaining in the luminal epithelial and tumor cell compartments. Scale bar, 50 μm. ***B.*** Relative SRC-3 mRNA levels in PtenCKO and Pten3CKO prostates

with tumors. Total RNA samples (n=8 for each group) were used in real time RT-PCR measurements. The mRNA level was normalized to β-actin mRNA level in the same sample. *, $p<0.05$ by Student's t-test. ***C.*** SRC-3 knockout significantly reduced prostate weight at 18 and 24 weeks of age. The relative prostate weight was obtained by normalizing to body weight. Ten mice were used for each age stage for each genotype group. *, $p<0.05$ and **, $p<0.01$ by Student's t-test. ***D.*** Gross picture showing the size difference between PtenCKO and Pten3CKO tumors at 18 weeks and H&E stained sections comparing the histology between the two groups. AP, DP, LP and VP, anterior, dorsal, lateral and ventral prostates; scale bar, 100 μm.

Fig. 3. Deletion of SRC-3 decreases tumor proliferation and changes cellular composition of the tumor. ***A.*** Ki67 immunohistochemistry (brown color) on prostate tumor sections of 18-week-old PtenCKO and Pten3CKO mice. Scale bar, 50 μm. ***B.*** Quantitative analysis of Ki67(+) cells (IHC is shown in Panel A) and p63(+) cells (IHC is shown in Panel C) in PtenCKO and Pten3CKO prostate tumors (n=6 per group). The percentage of Ki67(+) and p63(+) cells were calculated by dividing the number of positive cells by the number of total cells per visual field. *, $P<0.05$ and **, $p < 0.01$ by Student's t-test. ***C.*** Immunostaining of p63 basal cell marker (brown), K8 LEC marker (green) and K5 basal and precursor cell marker (red) as well as AR LEC marker (brown) on prostate tumor sections of 18-week-old PtenCKO and Pten3CKO mice. Scale bar, 50 μm. ***D.*** Quantitative analysis of percent K8(+)-, K5(+)- and AR(+)-staining areas to total areas on prostate tumor sections prepared from 18-week-old PtenCKO and Pten3CKO mice (n=6). The respective areas were measured using the NIH ImageJ software. *,

$p<0.05$ by Student's t-test.

Fig. 4. Castration increases tumor aggressiveness and cellular proliferation in PtenCKO mice. ***A.*** In PtenCKO mice, Pten deletion started at 2 weeks of age, which induced prostate tumor initiation and progression as indicated. PtenCKO mice were castrated at 12 weeks of age and analyzed at 18 weeks of age. ***B.*** Analysis of proliferating cells by Ki67 IHC and assessment of tumor histology by H&E staining in prostate tumors of non-castrated and castrated PtenCKO mice. The percentages of Ki67(+) cells in each group and the statistical analysis results are indicated. ***C.*** Trichrome staining of collagen (blue) and IHC of SMA and CD31 (brown) for assessment of reactive stroma in prostate tumors of non-castrated and castrated PtenCKO mice. For quantitative analysis indicated below each panel, stained areas and total areas were measured in 9 independent samples using NIH ImageJ software for statistical analysis. ***D.*** Immunohistochemistry of AR, p63 and E-Cadherin in prostate tumors of non-castrated and castrated PtenCKO mice. The decreased AR, increased p63 and decreased E-cadherin staining in tumors of castrated PtenCKO mice signify a de-differentiated phenotype. Quantitative analyses of the percentages of AR(+) cells, p63(+) cells and E-cadherin(+) areas in tumors of non-castrated and castrated PtenCKO mice (n=9 per group) are indicated. All scale bars in panels B–D, 100 μm.

Fig. 5. Deletion of *SRC-3* significantly reduces tumor size and cellular proliferation in CRPC. ***A.*** PtenCKO and Pten3CKO mice were castrated at 12 weeks of age and their prostates were imaged at 18 weeks of age. Their anterior prostate weights were measured

and normalized to their body weights. *, $p<0.05$ by Student's t-test, n=8 per group. ***B.*** SRC-3 and Ki67 IHC (brown). SRC-3 was present in the prostate tumor cells of castrated PtenCKO mice, but absent in the tumor cells of castrated Pten3CKO mice. The percentage of Ki67(+) cells was significantly higher in prostate tumors of castrated PtenCKO mice (n=6) versus castrated Pten3CKO mice (n=6). *, $p<0.05$ by Student's t-test. Scale bar, 50 μm. ***C*** and ***D.*** Deletion of *SRC-3* reversed castration-induced changes in tumor cell type and stromal reactivity. Many SRC-3(+) cells (green) including tumor cells and non-tumor basal and stromal cells were observed in PtenCKO tumors, but less number of SRC-3(+) non-tumor basal and stromal cells were observed in Pten3CKO tumors. Many more p63(+) cells (red) were observed in PtenCKO tumors versus Pten3CKO tumors, and many of the p63(+) cells in PtenCKO tumors co-expressed SRC-3 as evidenced by yellow color after merging the green and red colors in the same cells (upper panels in C). AR immunostaining (brown) was increased in Pten3CKO tumors versus PtenCKO tumors (medial panels in C). SMA (red) was detected in the stromal layer surrounding the PIN lesions of Pten3CKO mice, while SMA(+) stromal cells are disorganized in PtenCKO tumors. Many vimentin(+) cells (Green) were detected in PtenCKO tumors but not in Pten3CKO tumors (lower panels in C). Trichrome-stained collagen (blue) was abundant in PtenCKO tumors but little in Pten3CKO tumors (upper panels in D). E-cadherin immunostaining (brown) was higher in Pten3CKO tumors versus PtenCKO tumors (medial panels in D). The number of CD31(+) endothelial cells (arrows) were decreased in Pten3CKO tumors versus PtenCKO tumors (lower panels in D). All indicated quantitative results for immunostaining signals were calculated from positively stained areas versus total areas measured by ImageJ software. Scale bars in C

and D, 100 μm.

Fig. 6. SRC-3 deletion down-regulates Akt-mTOR signaling in CRPC tumors. ***A.*** Western blot analysis of phospho-Akt and total Akt levels in prostate tumors of castrated PtenCKO and Pten3CKO mice. ***B***. IHC for pRPS6 (brown) in prostate tumors of castrated PtenCKO and Pten3CKO mice. Scale bar, 50 μm. The percentages of pRPS6(+) areas to total areas were determined from 6 samples in each group. **, $p < 0.01$ by Student's t test. ***C.*** Western blot analysis of indicated protein levels in prostate tumors of castrated PtenCKO and Pten3CKO mice. ***D.*** Western blot analysis of indicated protein levels in PC3 human prostate cancer cells transfected with non-targeting control siRNA or SRC-3-knockdown siRNAs. Note the significant declines of p85S6K1 and pRPS6 in SRC-3-knockdown cells. ***E.*** The significant decrease in the S6K1 mRNA expression in Pten3CKO versus PtenCKO tumors from castrated mice. *, $p<0.05$ by Student's t-test, n=6 per group. ***F.*** SRC-3 expression positively correlates with S6K1 expression in human CaP samples (n=185). Data was downloaded from Oncomine database as indicated.

Fig. 7. Targeting SRC-3 in CRPC tumors altered some of the AR target gene expression. ***A.*** The expression of the AR-activated gene *Fkbp5* in prostate tumors of castrated Pten3CKO mice (n=6) is significantly higher than that in prostate tumors of castrated PtenCKO mice (n=6). ***B.*** The expression of AR-suppressed genes *Ets2* and *Igfbp3* in prostate tumors of castrated Pten3CKO mice (n=6) is significantly lower than that in prostate tumors of castrated PtenCKO mice (n=6). ***C.*** A broader panel of AR-

repressed genes was measured in the tumors of castrated PtenCKO and Pten3CKO mice (n=6). In all panels, the mRNA levels were measured by real time RT-PCR. *, $p<0.05$ and **, $p<0.01$ by Student's t-test.

EXPERT OPINION

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Steroid receptor coactivator-3 as a potential molecular target for cancer therapy

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Introduction: Steroid receptor coactivator-3 (SRC-3), also called amplified-in-breast cancer-1 (AIB1), is an oncogenic coactivator in endocrine and non-endocrine cancers. Functional studies demonstrate SRC-3 promotes numerous aspects of cancer, through its capacity as a coactivator for nuclear hormone receptors and other transcription factors, and via its ability to control multiple growth pathways simultaneously. Targeting SRC-3 with specific inhibitors therefore holds future promise for clinical cancer therapy.

Areas covered: We discuss critical advances in understanding SRC-3 as a cancer mediator and prospective drug target. We review SRC-3 structure and function and its role in distinct aspects of cancer. In addition, we discuss SRC-3 regulation and degradation. Finally, we comment on a recently discovered SRC-3 small molecular inhibitor.

Expert opinion: Most targeted chemotherapeutic drugs block only a single cellular pathway. In response, cancers frequently acquire resistance by upregulating alternative pathways. SRC-3 coordinates multiple signaling networks, suggesting SRC-3 inhibition offers a promising therapeutic strategy. Development of an effective SRC-3 inhibitor faces critical challenges. Better understanding of SRC-3 function and interacting partners, in both the nucleus and cytosol, is required for optimized inhibitor development. Ultimately, blockade of SRC-3 oncogenic function may inhibit multiple cancer-related signaling pathways.

Keywords: AIB1, cancer, gene expression, molecular target, nuclear receptor coactivator, SRC-3

Expert Opin. Ther. Targets [Early Online]

1. Introduction

Nuclear hormone receptors (NRs) bind DNA at specific sites to regulate gene expression and impact many physiological processes. Upon binding to its ligand, a NR commonly undergoes a conformational change and forms a dimer. The liganded NR complex then translocates into nucleus, recognizes specific regulatory DNA sequences, and binds response elements upstream of target genes to activate gene transcription. By themselves, NRs cannot initiate optimal transcriptional activation. It is through interaction with coactivator proteins that hormone-activated NRs can direct the assembly and stabilization of a preinitiation complex that ultimately conducts the transcription of the target genes [1]. The p160 steroid receptor coactivator (SRC) family, consisting of three members (SRC-1 [2]/NCOA1, SRC-2/TIF2 [3]/GRIP1 [4]/NCOA2, and SRC-3/p/CIP [5], RAC3 [6], AIB1 [7], ACTR [8], TRAM1 [9] and NCOA3), is a key coactivator group for serving as a bridge between the hormone-activated NRs, other co-regulators, and the basal transcriptional machinery. These SRC proteins can also act as coactivators for non-NR transcription factors to regulate target gene transcription and impact multiple growth factor pathways. In general, SRC proteins sit at the nexus of multiple cancer signaling pathways that impact cancer initiation, growth, migration,

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Article highlights.

- SRC-3 has been linked to various aspects of carcinogenesis ranging from cancer initiation, progression, cell motility and invasion, inflammation, angiogenesis and resistance to chemotherapy.
- SRC-3 can undergo post-translational modification (PTM) and PTM is responsible for its diverse functions.
- SRC-3 promotes cancer by activating nuclear receptors (NRs) such as ER and AR and facilitating transcription of multiple transcription factors. Thus SRC-3 sits at the nexus of multiple cellular pathways to promote cancer.
- SRC-3delta4, a splicing isoform of SRC-3, has been identified to play an important role in cell invasion and metastasis.
- SRC-3 can be regulated at the mRNA level by transcriptional regulation and microRNA and at protein levels by protein degradation pathways. Knowledge of SRC-3 regulation provides insights into designing small molecules for inhibiting SRC-3 function.
- Gossypol was recently identified to be an SRC-3 inhibitor that can degrade SRC-3 at the protein level.

This box summarizes key points contained in the article.

invasion, metastasis and chemotherapeutic resistance. In this review, we will focus on SRC-3 and discuss the role of SRC-3 in these distinct aspects of carcinogenesis. We will then evaluate the advantages and feasibility of designing SRC-3 inhibitors as therapeutic agents for cancer.

2. Steroid receptor coactivator-3

Steroid receptor coactivator -3(SRC-3) was initially found to be amplified and overexpressed in human breast cancer cell lines and a subset of breast tumors [10]. AIB1 (SRC-3) was identified during a search on the long arm of chromosome 20 for genes with overexpression and increased copy number in breast cancers [10]. Serving as an adapter to recruit chromatin remodeling proteins and other transcriptional enzymes, SRC-3 is known to mediate transcriptional activities of NRs such as ER (estrogen receptor) and PR (progesterone receptor). SRC-3 was found to promote hormone-dependent growth of human MCF-7 breast cancer cells by coactivating ERα and PRβ [11]. SRC-3 also potentiates the transcriptional activities of other TFs (transcription factors) such as E2F-1, PEA3, AP-1 and NF-κB (nuclear factor-kappa B) [12-15]. SRC-3 shares a common structure with the other members of the p160 family, containing domains with well-studied functional relevance. The structural and functional domains of SRC-3 are summarized in **Figure 1**. The N-terminal region is the most conserved among p160 family members and consists of bHLH (basic helix-loop-helix) region and a PAS (Per/ARNT/Sim) motif [16,17]. This region is necessary for several protein–protein interactions, including association with TFs such as myogenin and MEF2C [18-20]. In addition, the bHLH-PAS region contains multiple nuclear localization signals [21]. A region between the bHLH-PAS domain has been linked to the turnover and degradation of SRC-3 [22]. The central region of the SRC proteins contains three LXXLL (L, Leucine; X, any amino acid) motifs which form amphipathic α-helices and are essential for direct interactions with NRs in a ligand-dependent manner [23-26]. Finally, the C-terminal region contains two transcriptional activation domains (AD1 and AD2). The AD1 domain directly binds to CBP (CREB-binding protein) and p300 proteins [27]. The recruitment of CBP/p300 to the chromatin by SRC proteins results in histone acetylation necessary for SRC-mediated transcriptional activation. The AD2 domain interacts with histone methyltransferases such as CARM1 (coactivator-associated arginine methyltransferase 1) and PRMT1 (protein arginine N-methyltransferase 1) to promote histone methylation and subsequently facilitate chromatin remodeling [28,29]. Interestingly, the C termini of SRC-1 and SRC-3 also contain HAT (histone acetyltransferase) activity [30]. These structural elements allow SRC proteins to provide a platform through which transcription factors can interact with additional coregulators that promote chromatin remodeling and assembly of general transcription machinery. In sum, their structure underlies the ability of SRC proteins to coordinate signals from a myriad of cellular signaling pathways in regulating gene expression output.

SRC proteins exist at limited concentrations in normal physiology. They are considered "master regulators" of differential gene expression and accomplish this through combinatorial codes of post-translational modifications (PTMs). Known SRC-3 PTMs, the responsible modifying proteins, and the modified sites are listed in **Table 1**. Extracellular stimuli such as hormones, growth factors and cytokines activate signaling pathways that may post-translationally modify SRCs through phosphorylation, ubiquitylation, sumoylation, etc. The combinatorial codes of PTMs determine protein stability, interaction specificity and transcriptional activity of SRCs. Deregulation of these PTMs has a significant impact on cellular physiology and results in human diseases such as cancer. Early observation that SRC-3 localization and transcriptional activity could be regulated by IKKβ (IκB kinase β) phosphorylation provided the initial clue that SRC-3 is subject to PTM [31]. SRC-3 contains at least eight specific phosphorylation sites [32]. Seven Serine/Threonine (Thr24, Ser 505, Ser 543, Ser 601, Ser 857, Ser 860, and Ser 867) phosphorylation sites and one Tyr (Tyr 1357) phosphorylation site have been demonstrated to be functionally important [33,34]. These sites are phosphorylated by a number of different kinases including MAPK, IKK, GSK3α, GSK3β, and CK1d. SRC-3 is also a target of ABL tyrosine kinase which can be activated by estrogen and growth factors. Phosphorylation of Tyr1357 on SRC-3 by ABL tyrosine kinase increases binding of SRC-3 to p300 and transcription factors, thus mediating ER, PR, and NF-κB-dependent transcription activities [34]. Furthermore, Tyr1357 phosphorylation has been shown to increase in ERBB2-induced breast tumors in mice, suggesting an oncogenic function of SRC-3 with Tyr 1357 phosphorylation. In conclusion,

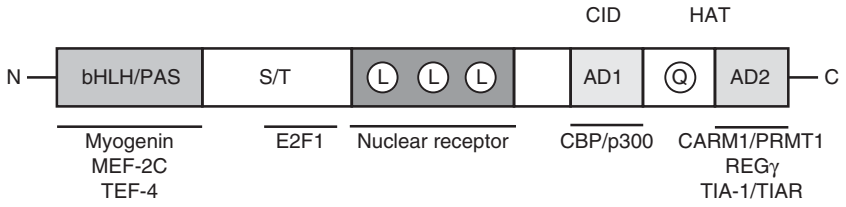

Figure 1. Structural and Functional Domains of SRC-3 protein. The letters within the bar figure indicates functional domains. AD1 contains CID (CBP/p300 interacting domain) and AD2 contains HAT (histone acetyltransferase domains). The proteins listed below the bar figure are known to interact with SRC-3. This list is incomplete.

AD1: Activation Domain 1; AD2: Activation Domain 2; bHLH: Basic helix-loop-helix domain; L: LXLL α-helix motif; PAS: Per/ARNT/Sim homologous domain; Q: Glutamine-rich domain; S/T: Serine/threonine-rich region.

Table 1. SRC-3 is subject to multiple post-translational modifications.

PTM	SRC-3 Modifier	Effect on SRC-3	Modified Residue	Ref.
Acetylation	CBP/p300	Inactivation	K626, K629, K630	[89]
Dephosphorylation	PP2A	Inactivation	S505, S543	[22]
	PP1	Stabilization	S101, S102	[22]
Methylation	CARM1	Inactivation, then Degradation	R1171	[90]
Phosphorylation	JNK	Activation	T24, S505, S543, S860, S867	[32]
	ERK	Activation	S505, S543	[32]
	p38MAPK	Activation	T24, S505, S543, S860, S867	[32]
	PKA	Activation	S857	[32]
	c-Abl	Activation	Y1357	[34]
	IKK	Activation	S857	[32]
	GSK3β	Activation, then Degradation	S505	[77]
	aPKC	Stabilization	S/T sites in a.a. 1031-1130 region	[91]
Ubiquitinylation	E6-AP	Degradation	Unknown	[78]
	$SCF^{Fbw7\alpha}$	Degradation	K723, K786	[77]
	CUL-3 and RBX1	Degradation	Unknown	[80]
	CHIP	Degradation	Unknown	[81]
	SPOP	Degradation	N-terminal	[83]
SUMOylation	SUMO-1	Inactivation	K731	[92]

Created by proteins from multiple intracellular signaling pathways, these modifications constitute a combinatorial code through which SRC-3 can integrate pathway-specific information to coordinate cellular outcomes.

aPKC: Atypical protein kinase C; c-Abl: Abelson tyrosine kinase; CARM1: Coactivator-associated arginine methyltransferase 1; CHIP: Carboxyl terminus of Hsc70-interacting protein; CUL-3: Cullin-3; E6-AP: E6-associated protein; ERK: Extracellular-signal-regulated kinase; Fbw7a: F-box and WD repeat domain-containing 7; GSK3β: Glycogen synthase kinase 3; IKK: IκB kinase; JNK: C-Jun N-terminal kinase; p38MAPK: P38 mitogen-activated protein kinases; PKA: Protein kinase A; PP1: Protein phosphatase 1; PP2A: Protein phosphatase 2A; RBX1: RING box protein 1; SCF: SKP1–cullin-1–F-box; SPOP: Speckle-type POZ protein.

phosphorylation can convert inactive SRC-3 into a potent transcriptional coactivator, resulting in differential gene expression with relevance to the development of cancer.

3. SRC-3 impacts multiple axes in cancer

The role of SRC-3 as a master regulator in cellular growth and development places it at the nexus of several intracellular signaling pathways critical for cancer (**Figure 2**). As SRC-3 amplification and overexpression have been correlated in multiple clinical studies with tumor aggressiveness or poor patient outcome (**Table 2**), it has become imperative to perform detailed mechanistic studies on the role of SRC-3 in tumorigenesis and cancer metastasis.

3.1 SRC-3 in cancer initiation and tumorigenesis

The proliferative role of SRC-3 in primary tumor formation has been extensively studied. Many mouse models have been employed to investigate the function of SRC-3 in cancer, particularly breast cancer. In transgenic mouse models where overexpression of SRC-3 in mammary epithelial cells was driven by MMTV (mouse mammary tumor virus) promoter, mammary hyperplasia and spontaneous development of mammary tumors were observed, directly supporting the role of SRC-3 in breast cancer initiation [35]. Hyperactive IGF-1 signaling was found in these MMTV-SRC-3 transgenic mice [35]. Consistent with results in this model, when SRC-3 knockout mice were crossed with MMTV-v-ras transgenic mice, mammary tumor incidence and growth rate were reduced dramatically in

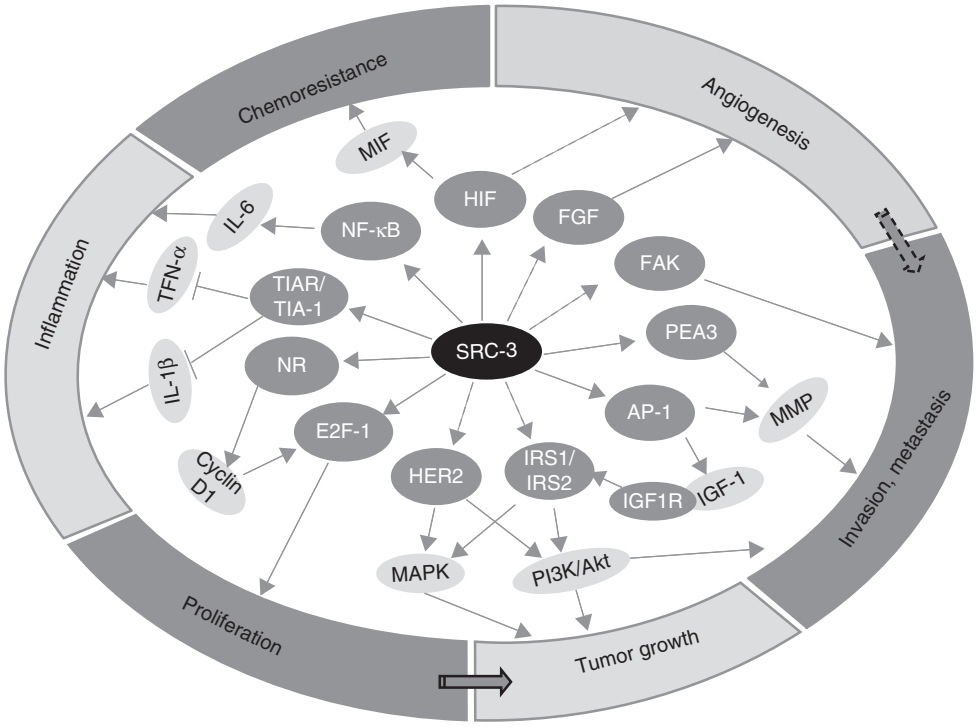

Figure 2. SRC-3 integrates multiple signaling pathways. Depending on the cellular context, SRC-3 can coactivate different nuclear receptors and transcription factors to promote multiple hallmarks of cancer. SRC-3 classically facilitates the transcriptional activities of nuclear receptors to promote cell growth. It also coactivates E2F-1 to promote cell division. To impact on tumor expansion, SRC-3 serves as a coactivator for AP1 to upregulate IGF-Akt pathway and may be involved in HER2 pathway. SRC-3 also acts as a pro-metastatic factor by coactivating PEA3 and AP1 to upregulate MMP production, a requisite process in extracellular matrix breakdown that accompanies invasive tumor behavior. A spliced SRC-3 isoform located in cytoplasm can also contribute to metastasis by serving as an adaptor for FAK-Src signal pathway. SRC-3 has been implied to bind NFκB and upregulate MIF through the HIF transcription factor. This, in turn, contributes to chemoresistance, as well as angiogenesis through the FGF signaling pathway. NR, nuclear receptor such as ER, PR or AR. The solid arrow indicates direct interaction or known pathways while the dotted arrow indicates indirect interaction.

SRC-3-/-;MMTV-v-ras bigenic virgin mice and inhibited completely in ovariectomized SRC-3-/-;MMTV-v-ras bigenic mice [36]. Further molecular analysis indicated SRC-3 deficiency did not alter estrogen and progesterone-responsive gene levels, but decreased IRS-1 (insulin receptor substrate-1) and IRS-2, resulting in impaired IGF-1 signaling pathway [36]. Along the same lines, ablation of SRC-3 in mice treated with chemical carcinogen DMBA (7,12-dimethylbenz[α]anthracene) protected against mammary gland tumorigenesis [37]. Another mouse model was used to assess the impact of SRC-3 ablation on MMTV-Erbb2 (erythroblastosis oncogene B 2)-induced mammary tumors. Knockout of SRC-3 in MMTV-Erbb2 mice completely suppressed tumorigenesis and reduced levels of phosphorylated ERBB2, cyclin D1, and cyclin E [38]. All these findings suggest that SRC-3 plays an important role in tumor initiation and growth and that targeting SRC-3 can effectively suppress tumor initiation and growth.

In addition to the full-length SRC-3, a splice variant of SRC-3 (SRC-3delta4) which lacks the N-terminal bHLH-PAS domain has been identified and found to be an even more potent coactivator for ER and PR than the full-length SRC-3 [39]. Transgenic mice overexpressing SRC3delta4 develop mammary gland hyperplasia, increased cyclin D1 expression, and increased IGF-1 receptor level [40]. Furthermore, combined overexpression of SRC3delta4 and ERα in mouse mammary gland resulted in increased incidence of hyperplasia and adenocarcinoma with increased stromal collagen deposition [41]. SRC-3delta4 also plays an important role in cancer metastasis and its function will be discussed in detail in a later section.

Besides breast cancer, SRC-3 was also found to be important for tumorigenesis in the prostate. SRC-3 knockout mice were crossed with TRAMP (transgenic adenocarcinoma mouse prostate mice), a mouse prostate cancer model. Total ablation of SRC-3 in TRAMP mice arrested prostate tumor growth at well-differentiated stages. Even though initiation of prostate tumors in this mouse model was not delayed, the progression of prostate tumorigenesis substantially declined [42].

Table 2. SRC-3 overexpression and amplification are associated with aggressive tumor phenotype or poor prognosis in human carcinomas of endocrine and non-endocrine organs.

Cancer Type	Change	Detection	Frequency	Clinical Associations	Ref.
Endocrine carcinomas					
Breast	Gene amplification	FISH	9.50%	Undetermined	[10]
	mRNA Overexpression	ISH	64%	Positive correlation between expression and tumor size	[10]
	Protein Overexpression	WB	25%	Positive correlation between expression and tamoxifen resistance	[62]
	Protein Overexpression	WB	25%	Highly correlated with proliferation and expression of ERBB2 and PR	[62]
	Protein Overexpression	IHC	73.8%	Positive correlation between expression and MMP2, MMP9, and PEA3 protein levels	[13]
Prostate	mRNA Overexpression	ISH	ND	Positive correlation between expression and tumor grade	[93]
	Protein Overexpression	IHC	ND	Positive correlation between expression and tumor grade	[93,94]
Endometrial	mRNA Overexpression	qPCR	17%	Undetermined	[95]
	Protein Overexpression	IHC	97%	Associated with poor prognosis	[96]
Ovarian	Gene Amplification	FISH	25%	Associated with DNA amplification at 20q12-q13	[97]
	Protein Overexpression	IHC	64%	expression of SRC-3 positively correlates with invasiveness of tumor	[44]
Non-endocrine carcinomas					
Esophageal squamous cell carcinoma	Gene Amplification	FISH	4.3 – 4.9%	Associated with chromosome 20q amplification and increased copy number	[98,99]
	Protein Overexpression	IHC	46%	Associated with large tumor size and Ki67 proliferation staining	[99]
Gastric	Gene Amplification	FISH	7%	Amplification positively	[100]
	mRNA Overexpression	Northern, qPCR	40%	correlated with metastasis	[100]
Colorectal	Gene Amplification	FISH	10%	Nuclear expression positively	[101]
	mRNA Overexpression	IHC	35%	correlated with metastasis	[101]
Pancreatic	Gene Amplification	FISH	37%	Undetermined	[102]
	mRNA Overexpression	ISH	> 65%		[102]
	Protein Overexpression	IHC	> 65%		[102]
Hepatocellular	Gene Amplification	FISH	25%	Undetermined	[103]
	Protein Overexpression	WB	68%	Undetermined	[104]
Urothelial	Gene Amplification	FISH	7%	Undetermined	[105]
	Protein Overexpression	IHC	33%	Overexpression positively correlated with Ki67 proliferation staining	[105]
Nasopharyngeal	Gene Amplification	FISH	7%	Overexpression positively associated with large tumor and Ki67 index	[106]
	Protein Overexpression	IHC	51%	Undetermined	[106]

FISH: Fluorescence *in situ* hybridization; IHC: Immunohistochemistry; ISH: In situ hybridization; qPCR, quantitative PCR; WB: Western blot.

3.2 SRC-3 in cell motility, invasion, and metastasis

In order for metastasis to manifest, cancer cells need to gain motility and invasive potential that will allow them to escape the primary tumor site, invade surrounding stroma and enter the blood stream. The first evidence that SRC-3 plays a role in cell migration and invasion comes from the studies of fruit fly ovary [43]. In the absence of Taiman, the Drosophila homolog of SRC-3, ecdysone receptor-dependent border cell motility and invasiveness were markedly suppressed and there was an abnormal cellular build-up of E-cadherin, β-catenin, and focal adhesion complexes [43]. Subsequently, in human ovarian cancer cells, SRC-3 was demonstrated to be important for cellular spreading migration on the substratum [44]. The most relevant *in vivo* study of SRC-3 function in metastasis originates from MMTV-polyoma middle T antigen (PyMT) transgenic mouse model. Absence of SRC-3 in PyMT

transgenic breast cancer mouse model significantly suppressed mammary tumor metastasis to the lung [13]. Molecular studies showed that SRC-3 could impact the expression levels of MMPs (matrix metalloproteinases) that allow tumor cells to break down the extracellular matrix and invade into stromal compartment. In both human (MDA-MB-231) and PyMT tumor cells in culture, SRC-3 regulates MMP2 and MMP9 by directly binding and potentiating activity of the PEA3 transcription factor [13]. SRC-3 also serves as a coactivator for AP-1 (activator protein 1) to drive expression of MMP7 and MMP10 in MDA-MC-231 human breast cancer cells [45]. Furthermore, in prostate cancer, SRC-3 simultaneously coactivates AP-1 and PEA3 to upregulate expression of MMP2 and MMP13 [15]. By serving as a coactivator for a number of TFs responsible for the expression of MMP family members, nuclear SRC-3 promotes invasion of cancer cells into the surrounding stromal compartment. Recently, in a lung cancer cell line, ERK3 was shown to phosphorylate SRC-3 at S857, a modification essential for the binding of SRC-3 with PEA3 and promotion of MMP gene expression [46]. In sum, SRC-3 clearly promotes cancer invasion by coactivating non-nuclear receptor TFs to regulate MMP gene expression.

SRC-3delta4 also has been implicated in promotion of metastasis. SRC-3delta4 is mainly sequestered in the cytosol and acts as a signal adaptor for EGFR (epidermal growth factor receptor) and FAK (focal adhesion kinase-1) at the plasma membrane [47]. EGF is a critical mediator for cancer cell migration and metastasis [48]. Extracellular EGF binds EGFR on the cell membrane and activates a number of intracellular protein kinases including PAK1 (p21-activated kinase 1) [49], FAK [50] and c-Src [51] in a cascade signaling fashion. SRC-3delta4 serves as a bridge between EGFR and FAK to allow optimal activation of EGF-FAK-cSrc signal transduction [47]. Activation of this signal pathway promotes the movement and invasion of cancer cells.

3.3 SRC-3 in inflammation and angiogenesis

Persistent inflammation is a characteristic of the tumor microenvironment and is recognized as a hallmark of cancer. Inflammation can promote proliferation and survival of cancer cells, facilitate angiogenesis and metastasis, destabilize adaptive immunity, and reduce response to hormone therapy and chemotherapy [52]. As in other inflammatory contexts, accumulating evidence shows NF-κB is a key mediator of tumor inflammation. SRC-3 has been shown to interact with and coactivate NF-κB in HeLa cancer cells [14]. In response to TNF-α (tumor necrosis factor–α), SRC-3 is phosphorylated by IKK in cytosol of HeLa cells [31]. SRC-3 translocates along with NF-κB into the nucleus where, aided by SRC-3, NF-κB can bind promoters of target genes and promote the initiation of inflammatory responses. One important NF-κB target gene is IL-6 (interleukin-6), a pro-inflammatory cytokine that plays an important role in tumor metastasis and inflammation [53]. IL-6 is elevated in prostate cancer tissues and acts as an autocrine growth factor in prostate cancer [54,55]. This suggests SRC-3 can serve as a coactivator for NF-κB to promote inflammation in cancer. Demonstrating the context specificity of inflammatory responses, SRC-3 knockout mice are actually more susceptible to acute inflammatory responses than controls [56]. SRC-3 knockout macrophages are more sensitive to LPS (lipopolysaccharide)-induced endotoxic shock and they produce more pro-inflammatory cytokines. In these cells, SRC-3 was demonstrated to bind translational repressors TIA-1 (T cell intracellular antigen-1) and TIAR (TIA1-related protein) to inhibit translation of TNF-α, IL-6, and IL-1 [56]. In addition, SRC-3 is required for clearing bacteria, and represses inflammatory response in E. Coli-induced septic peritonitis [57]. These findings suggest that the role of SRC-3 in NF-κB-mediated cytokine expression may be specific to cell types.

Angiogenesis is another important process in cancer progression because the growth of a tumor relies on a sufficient blood supply. Many studies have been focused on investigating the function of SRC-3 in growth factor signaling, with the main focus on cell-autonomous regulation of proliferation and invasive capacity. However, less is known about the SRC-3 function in stroma. A recent study elucidates the role of SRC-3 in angiogenesis and wound healing [58]. SRC-3 was shown to promote proliferation and motility of endothelial cells, such that neoangiogenesis was dependent on the presence of SRC-3 [58]. The study also demonstrated that both alleles of SRC-3 were required for proper wound healing *in vivo* and that SRC-3 may cross-talk with FGF (fibroblast growth factor) signaling to regulate wound healing process [58].

The tumor microenvironment plays a critical role in cancer progression. By immunohistochemistry, SRC-3 protein expression is found in stromal compartment. However, the *in vivo* function of SRC-3 in tumor microenvironment has not been clearly defined due to lack of appropriate models. Future investigation of SRC-3 in tumor microenvironment can be aided by the generation of mice with floxed SRC-3 alleles [59], so that SRC-3 may be deleted in specific cell types with relevance to these novel functions.

3.4 SRC-3 in endocrine therapy-resistant cancer and chemoresistant cancer

SRC-3 has been implicated in the development of resistance to chemotherapeutic agents. Tamoxifen is an antagonist that competes with estrogen for binding to ER, resulting in the inhibition of ER-mediated transcription and thus estrogen dependent cancer growth. Tamoxifen has been the standard endocrine therapy for women with ER-positive breast cancer. However, only 50% of ER-positive breast cancer patients respond to tamoxifen therapy [60]. Other patients treated with tamoxifen for long periods tend to acquire resistance to the therapy. Resistance to endocrine therapy often has been associated with activation of growth factor signaling pathways such as EGFR pathway [61]. There is a positive correlation between SRC-3 protein expression and the levels of HER

family proteins in the breast cancer patients with recurrence after tamoxifen treatment [62]. Recently, it was demonstrated PAX2 (paired box gene 2) competes with SRC-3 for binding and regulation of HER2 transcription. High SRC-3 expression was associated with high recurrence rate in patients with ER-positive tumors and treated with tamoxifen [63]. Another class of endocrine therapeutic agent, aromatase inhibitors, acts by blocking conversion of testosterone and androstenedione into estrogen. Aromatase inhibitors are used to treat postmenopausal women with ERα-positive breast cancer. However, breast tumors with high HER2 and SRC-3 expression may also develop resistance to aromatase inhibitors, as its family member SRC-1 does [64].

Bortezomib (PS-314 or Velcade) is a proteasome inhibitor that has anti-cancer activity in various cancer cell lines including prostate cancer cell and prostate cancer xenograft models. In a neoadjuvant clinical trial of bortezomib in men with prostate cancer at high risk of recurrence, unexpected increase in proliferation in treated tissues and cultured cells was found [65]. In these treated tissues and cell lines, SRC-3 level and phosphorylated Akt level were found to be increased. Knockdown of SRC-3 decreased the level of the phosphorylated Akt. These data suggest that SRC-3 may contribute to chemo-resistant prostate cancer [65].

A recent paper identified MIF (macrophage migration inhibitory factor) as a new target gene of SRC-3 and demonstrated MIF is a suppressor of autophagic cell death [66]. Upregulation of MIF expression by SRC-3 in cancer cells can contribute to chemoresistance. Inhibition of MIF expression can sensitive cancer cells to anti-cancer drugs such as doxorubicin and etoposide [66].

4. SRC-3 in development, metabolism and other physiological process

Genetically engineered mouse models have been employed to study the physiological relevance of steroid receptor coregulators. Much of our understanding about SRC-3 function *in vivo* stems from characterization of SRC-3 knockout mice. Targeted deletion of SRC-3 in mice has revealed its critical role for normal somatic growth, mammary gland development and female reproduction [67,68]. Circulating IGF-1(insulin-like growth factor-1) level was found to be significantly reduced in SRC-3 knockout mice [69]. All three members of SRC family play a critical role in metabolic regulation. In particular, loss of SRC-3 impairs white adipogenic differentiation through decreased PPARγ2 (peroxisome proliferator-activated receptor-γ activity) [70]. SRC-3 knockout mice are resistant to high-fat diet-induced obesity and have improved insulin sensitivity [71]. The phenotype was partly due to the regulation of PGC-1 (peroxisome proliferator-activated receptor-γcoactivator-1) acetylation by SRC-3 [71]. Furthermore, a knock-in mouse model with mutations at four conserved phosphorylation sites displayed increased body weight and adiposity, and reduced peripheral insulin sensitivity. These mice were also more susceptible to carcinogen-induced liver tumorigenesis. These results support the idea that PTMs are important for the normal function of SRC-3 and that changes in PTMs are sufficient to alter glucose homeostasis and cancer susceptibility [72]. Because all cancer cells rely on changes in metabolism to support growth and survive, targeting metabolism for anti-cancer therapy has been a recent focus in cancer research. Owning to its close relevance to metabolism, the exploration on the function of SRC-3 in the regulation cancer metabolism might provide some insights into successful cancer therapy.

5. Regulation of SRC-3 mRNA/protein levels

SRC-3 expression and protein amount can be regulated at three different levels: Transcription, translation and protein degradation. SRC-3 is a coactivator for E2F-1 [12,73] and SRC-3 gene contains E2F-1 binding sites at its promoter region suggesting SRC-3 can self-regulate and form a positive feedback loop for its own expression [12]. Another binding site on the SRC-3 promoter region is for the SP1 (Specificity Protein 1) transcription factor. Interestingly, E2F-1-dependent transcription of SRC-3 did not require E2F-1 binding to its binding site but rather the binding of SP1 to the SP1 binding site in a proximal SRC-3 promoter region. At translational level, SRC-3 can be regulated by miRNAs (microRNAs). Endogenous miRNAs bind on site-specific sequences within the 3-untranslated regions and inhibit the translation. miRNA Mir-17-5p was found to specifically inhibit the translation of SRC-3 mRNA. In breast cancer cell lines with high levels of SRC-3 protein, Mir-17-5p was found to be at low levels [74].

Specific post translational modifications such as phosphorylation and methylation serve as a code that mediates SRC-3 interaction, function and degradation [75]. SRC-3 protein turnover is mediated by proteasomal degradation pathways [76] and the NLS (nuclear localization signal) within the bHLH domain of SRC-3 is critical for this proteasome-dependent turnover [21]. In the ubiquitin-dependent proteasome degradation, ubiquitin molecules are linked to the target proteins by E3 ligases. The ubiquitinylated proteins are then degraded by the 26S proteasome in an ATP-dependent manner. Both $SCF^{Fbw7\alpha}$ and E6-AP are examples of E3 ligase that can interact with SRC-3, targeting SRC-3 for degradation [77,78]. REGγ, 20S proteasome regulator, can also interact with SRC-3 and mediates its turnover in an ubiquitin-independent manner [79]. Recently, the components of E3 ligase, CUL1 and RBX1, were shown to be involved in SRC-3 ubiquitinylation and degradation, in response to retinoid acid treatment [80]. CHIP (carboxyl terminus of Hsc70-interacting protein) is a U-box-type ubiquitin ligase that induces ubiquitinylation and degradation of its substrates. SRC-3 was also found to be a target of CHIP and knockdown of SRC-3 reduces Smad and Twist expression [81]. In human hepatocellular carcinoma, Hepatitis B virus X protein (HBx) stabilizes SRC-3 so SRC-3 cannot be targeted for degradation by E3 ligase [82]. Recently,

speckle-type POZ protein (SPOP), a cullin 3 (CUL3)-based ubiquitin ligase, was found to promote SRC-3 ubiquitinylation and degradation [83]. Interestingly, loss-of-function mutations of SPOP were identified in 6 – 13 % of human prostate cancers that do not contain PTEN mutation or TMPRSS2:ERG fusion rearrangement [84]. In addition, a recent study of exome sequencing in 112 human prostate tumor and normal tissue pairs identified SPOP as one of the most frequently mutated gene in 13% of prostate tumors and all the SPOP mutation affected in the structure that involves in substrate-binding function [85]. Thus, it would be interesting to find out whether SPOP mutation is associated with SRC-3 protein elevation and human prostate carcinogenesis.

6. Development of an SRC-3 inhibitor

A specific SRC-3 inhibitor has not yet been generated. This is largely due to an incomplete understanding of the protein's structure and lack of crystallography data. By employing high throughput screening assays, the O'Malley lab recently identified gossypol as a small molecule inhibitor of SRC-1 and SRC-3 [86]. Gossypol, a compound derived from the cotton plant, has been shown to partially inhibit SRC-3 function in cell culture, stemming the growth of cancerous but not non-cancerous cells. Gossypol selectively reduces SRC-1 and SRC-3 protein levels in cancer cell lines including breast, prostate, lung, and liver cells. In fact, gossypol could reduce cell viability while also sensitizing lung and breast cancer cell lines to other chemotherapeutic agents such as MEK (MAPK kinase) inhibitor and EGFR inhibitors. Identified as an inhibitor for Bcl-2, gossypol was already demonstrated to be a proapoptotic agent for cancer cells and is currently being evaluated as a therapeutic agent for prostate cancer and lung cancer in clinical trials [87,88]. O'Malley's group identified that gossypol could directly bind to SRC-3 and reduce its protein levels without affecting its mRNA level. They also demonstrated gossypol could reduce mRNA levels of Bcl2. To further explain, genomic study of SRC-3 cistrome in MCF-7 identified several SRC-3 binding sites within Bcl-2 and Bcl-X genes. Knockdown of SRC-3 in MCF-7 cells decreased both Bcl-2 mRNA and protein levels. These findings further support that the anti-cancer mechanism of gossypol is through down regulation of SRC-3 protein levels, impairing anti-apoptotic pathways in cancer cells.

In pre-clinical and clinical studies, gossypol has had mixed results as a chemotherapeutic agent in the treatment of serious malignancies like small cell lung and adrenal cancers. Although gossypol has its own weaknesses including *in vivo* toxicity to serve as an effective therapeutic cancer drug in human [87,88], the identification of gossypol to be a small molecule inhibitor of SRC-1 and SRC-3 is a proof-of-principle study that oncogenic coactivators can be directly targeted for inhibiting cancer growth. Taken together, gossypol studies suggest direct inhibition of SRC-3 by small molecular inhibitors is possible and has specific impact on cancerous cells. Nonetheless, the failure to achieve near complete SRC-3 inhibition, even in culture, indicates we do not yet understand the full potential for SRC-3 inhibition in modulating disease. Given promising results of SRC-3 knockout in animal models, it will be critical to search for improved inhibitors whose efficacy against real tumors can be evaluated.

7. Expert opinion

Most chemotherapeutic drugs have been designed to target one particular growth factor pathway. For examples, tamoxifen is an antagonist for ERα in breast cancer while Herceptin and Lapatinib target to inhibit ERBB2. Even though these drugs are effective initially, cancer cells eventually upregulate different growth factor pathways to acquire resistance. Therefore, it is important to rationally design a drug that targets multiple pathways simultaneously. SRC-3 is a potential therapeutic target that impacts multiple growth pathways. SRC-3, like its family members, has been shown to play important roles in many aspects of cancer. SRC-3 is an oncogene in that its overexpression is associated with cancer initiation, progression, invasion, metastasis, and chemoresistance. Detailed knowledge of transcription regulation mediated by SRC-3 has been acquired through many *in vitro* studies, while the functional relevance of SRC-3 in multiple cancer pathways has been illustrated *in vivo*. It is well established that SRC-3 functions as a coactivator for NR and promotes NR-dependent cell proliferation. SRC-3 can also affect NR-independent cancer motility and invasion by serving as a coregulator for other TFs, such as E2F-1, AP1 and PEA3. Functions of SRC-3 outside its capacity as a transcription regulator have also been demonstrated. SRC-3 was identified as a translational corepressor for TIA-1/TIAR, which inhibits the production of pro-inflammatory cytokines, while SRC-3delta 4 interacts with EGFR and FAK1 to regulate cell invasion and migration. Together, these findings suggest SRC-3 functions to promote many aspects of carcinogenesis and impact multiple cancer pathways (**Figure 2**). Furthermore, SRC-3 knockout mice have elucidated the physiological relevance of SRC-3 in multiple cancers and served as models for preclinical trials of SRC-3 inhibition. Genetic ablation of SRC-3 in both breast and prostate cancer mouse models inhibits tumorigenesis and blocks metastasis. Given the fact that some SRC-3 knockout mice survived through embryonic and neonatal stages and all survived beyond these early stages have a nearly normal life span, specific inhibition of SRC-3 function may be an ideal approach to control cancer growth without severe side effect.

Ideal therapeutic agents should selectively kill tumor cells while sparing surrounding normal cells. SRC-3 is present at limiting concentration in normal cells and overexpressed in cancer cells. Overexpression of SRC-3 in cancer cells provides a growth advantage, such that cancer cells become "addicted" to SRC-3. Therefore, an SRC-3 inhibitor can theoretically target cancer cells to a greater degree than normal cells. Inhibition of SRC-3 may upregulate cytokine production in some cells of

the innate immune, rendering increased risk of cytokine storm [56]. While altered immunity is a potentially serious side effect, the normal viability and health of SRC-3 knockout mice suggests SRC-3 inhibition may be relatively safe.

Many important questions remain to be addressed. First, SRC-3 functions outside transcriptional regulation appear multiple, but remain poorly understood. Second, while many *in vivo* studies of SRC-3 have been carried out in SRC-3 total knockout mice, the roles of SRC-3 in specific cell types such as epithelial vs. stromal cells remain to be investigated. It is important to utilize conditional knockout mice to study cell-specific functions of SRC-3 in carcinogenesis. Temporal deletion of overexpressed SRC-3 in mouse models may also provide insights as the stages of cancer progression for which SRC-3 is most relevant. In addition, clearly mapping genes regulated by SRC-3 is important. Even though a small molecule inhibitor of SRC-3 has been identified, better and improved inhibitors for SRC-3 are still necessary. To develop such inhibitors, a crystal structure of SRC-3 protein may be advantageous (although analysis of large proteins like SRC-3 in this manner is extremely challenging). Other detailed research on the regulation of SRC-3 function and degradation may also provide insights for rational design of SRC-3 inhibitors.

The ultimate goal of future research is to block SRC-3 oncogenic function and inhibit multiple cancer-related signaling pathways. However, better knowledge of structure, interaction partners and the manner in which these interaction partners change during cancer progression will be important areas of research.

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Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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